GENETIC ANALYSES OF NONMOTILE DOUBLE MUTANTS IN *SALMONELLA TYPHIMURIUM*: A NEW MAPPING METHOD BY ABORTIVE TRANSDUCTION[1]

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IN Salmonella, mutations in *mot* (motility) and *fla* (flagellation) genes cause paralysis and absence of flagella, respectively. These nonmotile mutants, when treated with phage P22 grown on a wild-type strain, produce swarms (motile complete transductants) and trails (motile abortive transductants: LEDERBERG 1956; STOCKER 1956) on a semisolid medium. Many nonmotile mutants have been classified into complementation groups by abortive transduction and mapped by linkage analyses using complete transduction, colicinoduction and Hfr crosses. Three *mot* complementation groups (*motA*, *motB* and *motC*) and eight *fla* groups (*flaA*, *B*, *C*, *D*, *E*, *J*, *K* and *L*) have been shown to be located near *H1* (the phase 1 flagellar antigen gene) (JOYS and STOCKER 1963, 1965, 1969; IINO and ENOMOTO 1966; ENOMOTO 1966a, 1966b; ENOMOTO and YAMAGUCHI 1969; YAMAGUCHI personal communication), and one group, *flaF*, between *trp* and *gal* (SMITH and STOCKER 1962). However, the order of the *mot* and *fla* cistrons near *H1* is not completely known. A $ah1^-$ mutation which causes inactivation of *H1* and maps at a site closely linked to it results in absence of flagella in cells in phase 1 (IINO 1961). Double mutants having a genetic constitution of mot^- fla^-, mot^- $ah1^-$, or mot^- mot^- can also produce swarms and trails in transduction, only if the transduced chromosome fragment can carry both of the wild-type alleles corresponding to the two mutations.

Several phenomena involving transformation, transduction or conjugation have been used for mapping in bacterial genetics. Four of them, abortive transformation (IYER 1965), abortive transduction (STOCKER, ZINDER and LEDERBERG 1953; OZEKI 1959), complementation by F-prime transfer (JACOB and MONOD 1961) and interrupted mating (WOLLMAN and JACOB 1955) do not depend on the relative frequency of recombination between the chromosome of donor and recipient. Abortive transduction, however, has usually been used for a *cis-trans* test, that is, "all or none" complementation test for identifying cistrons.

In the investigation presented here a number of double motility mutants have been isolated. The relative positions of their *mot* and *fla* genes have been mapped by ordinary recombination-dependent mapping methods and then, based on the order revealed, the frequency of abortive transductants produced by the double mutants has been shown to be useful for genetic mapping as a new tool requiring

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no recombination. How transducing fragments arise from bacterial chromosomes is discussed in relation to the new mapping method.

MATERIALS AND METHODS

Bacteria and phage: All of the mutants used in this work were derivatives of the wild-type strain of *Salmonella typhimurium*, LT2, also termed TM2. The mutants, *motA257*, *motB275* and *motC244*, used as parents for isolation of double mutants, and other *mot* and *fla* mutants used have been described (ENOMOTO 1966a; IINO and ENOMOTO 1966). Strain SJ697 in which *H1-i* has been replaced by *H1-gp* of *S. dublin*, was used for tests of cotransduction of *H1* with *mot* or *fla* genes. SJ1907 (*motB275 motC244*) and SJ1909 (*motA257 motC244*) were obtained by cotransduction of the *motC244* gene with ahl^+ to $motB^-$ ahl^- and $motA^-$ ahl^- recipients respectively, and SJ1910 (*motC244 H1-a*) was obtained by replacing *H1-i* of the *motC244* mutant by *H1-a* of *S. abortus-equi*, SJ241. These transductions were carried out by the method described previously (ENOMOTO 1969). Phage P22 was used for transduction (ZINDER and LEDERBERG 1952) and lysates were prepared by the agar-layer method (ADAMS 1959).

Media: The composition of nutrient broth, nutrient agar (NA), and semisolid nutrient gelatin agar (NGA) was presented previously (ENOMOTO 1966a).

Transduction method: This followed the methods described by ENOMOTO (1966a, 1967).

Isolation of mot fla *double mutants from* mot *parents:* Broth cultures of single-colony isolates from each of the three parental *mot* mutants were streaked on the NGA plates and incubated for 48–60 hr at 37°C. LP-type sectors, i.e., resembling the large, pale colonies characteristic of the *fla* mutants on this medium (ENOMOTO and IINO 1963) appeared at the margins of the confluent growth. One LP-type sector was picked from each broth culture, streaked on an NA plate and examined with antisera for the phase 1 flagellar antigen, i, and the phase 2 antigen, 1.2. Transduction was also carried out from each parental *mot* to these mutants. Those mutants which agglutinated with neither of the antisera and did not produce motile transductants (swarms) were regarded as *mot fla* double mutants resulting from a secondary spontaneous mutation in a *fla* gene.

RESULTS

Grouping of mot fla *double mutants by the number of abortive transductants:* A large number of LP-type sectors (ENOMOTO and IINO 1963) were isolated from cultures of the three parental *mot* mutants, *motA257*, *motB275* and *motC244*, on NGA medium. They were regarded as *mot fla* double mutants, because they did not agglutinate with antisera for the flagellar antigen i and 1.2, and produced neither swarms (complete transductants) nor trails (abortive transductants) in transduction from their *mot* parent. These mutants could be grouped according to the number of trails which they produced in transduction from the wild-type strain, TM2, using a constant input ratio of about 10. The number of trails was expressed as percent of the number produced by the parental *mot* as recipient in transduction from TM2. As *motA* and *motB* are very close together on the chromosome it is convenient to consider together double mutants derived from *motA* or from *motB*. Thirty-three mutants derived from *motA257*, and twenty-nine from *motB275* were divisible into four groups (Table 1). The group I mutants comprising 10 for *motA* and 5 for *motB* produced no trails. Group II comprised 15 *motA* and 16 *motB* mutants which produced between 0.02% and 1% of the control number of trails. Group III comprised 5 *motA* and 2 *motB* mutants which produced between 1% and 10% of the control. Group IV comprised 3 *motA* and

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TABLE 1

Grouping of mot fla *double mutants*

Parental *mot*	Group	Percent trail production	Number of mutants	Cistrons (Number of identified mutants)
	I	0 (<0.0006)	10	*flaF* (3), unknown (7)
motA257	II	0.02–1	15	*flaA* (10), *flaB* (1), unknown (4)
	III	1–10	5	*ah1* (3), unknown (2)
	IV	50–150	3	*flaC* (2), *flaK* (1)
	I	0 (<0.0006)	5	*flaF* (3), unknown (2)
motB275	II	0.02–1	16	*flaA* (13), *flaB* (2), *flaAB* (1)
	III	1–10	2	*ah1* (2)
	IV	50–150	6	*flaC* (4), *flaK* (2)
	I	0 (<0.0006)	10	*flaF* (7), unknown (3)
motC244	II	0.02–0.3	5	*flaC* (1), *flaK* (2) unknown (3)
	IV	50–150	37	*flaA* (10), *flaB* (1), *flaD* (1), unknown (25)

6 *motB* mutants which produced about as many trails as the parental *mot* strains. The 52 double mutants derived from *motC244* by the same criteria comprised: 10 group I mutants, giving no trails; 5 group II mutants, producing very few trails (less than 0.3% of the control value); and 37 group IV mutants, producing about as many trails as their parental strain, *motC244*.

Complementation tests with known fla *mutants: motC244 fla* group I (zero trail production): Ten double mutants were used as donors in transduction to 8 complementation tester strains; *flaA34*, *flaB36*, *flaC38*, *flaD42*, *flaE60*, *flaF25*, *flaJ28*, and *flaK44* (Joys and Stocker 1965; Iino and Enomoto 1966). Three of the ten complemented (i.e., produced trails in crosses with) all the tester strains. Seven did not complement or only partially complemented *flaF25* and complemented the remaining 7 testers. The results of transduction to *flaF25* and *flaF35* as recipients are shown in Table 2; 7 mutants are found to be *motC244 flaF* double mutants.

motC244 fla *group II (0.02–0.3% trail production):* The results of transduction using 5 double mutants as donors and 4 tester strains, *flaC*, *flaK*, *flaE* and *flaJ*, as recipients (Table 3) indicate that *motC fla-46* belongs to the *flaC* cistron,

TABLE 2

Transduction from group I mutants of motC244 fla *to* flaF

Recipient	Donor *motC244 fla⁻* 112	104	109	102	113	134	106	123	133	135	TM2
flaF25	2(0)	2(0)	0(0)	0(0)	2(0)	2(1)	2(1)	2(3)	2(3)	2(3)	3(3)
flaF35	2(1)	2(1)	2(1)	2(3)	2(3)	2(3)	2(3)	2(3)	2(3)	2(3)	2(3)

0.1 ml mixture of equal volumes of bacterial and phage suspensions (1×10^9 cells per ml, an input ratio of around 5) was inoculated, if necessary diluted to 10 or 100 times, on a NGA plate. Transductants of three plates were counted and converted to percent of the number produced by TM2. Numbers outside and inside of parentheses show degree of swarm and trail production respectively: grade 3, 50–150% production; grade 2, 1–50%; grade 1, less than 1%; 0 showing no production.

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TABLE 3

Transduction from group II mutants of motC244 fla *to the tester strains*

Recipient	Donor							
	flaC38	*motC244 fla-46*	*flaK44*	*motC244 fla-48*	*motC244 fla-49*	*motC244 fla-47*	*motC244 fla-126*	TM2
flaC38	0(0)	1(0)	3(3)	3(3)	3(3)	3(3)	3(3)	3(3)
flaK44	3(3)	3(3)	0(0)	1(1)	0(0)	3(3)	3(3)	3(3)
flaE60	3(3)	3(3)	2(2)	1(1)	3(3)	3(3)	3(3)	3(3)
flaJ28	3(3)	3(3)	2(2)	2(2)	3(3)	3(3)	3(3)	3(3)

Transduction procedures and abbreviations are the same as Table 2.

and *motC fla-48* to *flaK* or *flaE* and *motC fla-49* to *flaK*. The remaining two double mutants, *motC fla-47* and *motC fla-126*, complemented all the eight tester strains including those not shown in Table 3, i.e., *flaA, flaB, flaC, flaD, flaE, flaF, flaJ* and *flaK*. *flaK44* and *motC flaK48* showed only a low grade of complementation with *flaE60* and *flaJ28*. Complementation groups *E* and *J* are therefore perhaps subgroups of group *K*.

motC244 fla *group IV (50–150% trail production):* For complementation tests on this group the tester strains were used as donors and the double mutants as recipients. In transduction with the eight *fla* testers, *motC fla-114* and *motC fla-50* did not complement *flaB36* and *flaD42*, respectively. The remaining 35 mutants complemented all the eight tester strains except *flaA34*. A *flaA* complementation group can be divided into four subgroups by the grade of abortive transduction among the *flaA* mutants (IINO and ENOMOTO 1966). These subgroups are hereafter termed as follows: $flaA_1$ (represented by *flaA37*); $flaA_2$ (*flaA23* or *flaA34*); $flaA_3$ (*flaA22*); and $flaA_4$ (*flaA39*). $flaA_4 39$ is characterized as showing a low grade of complementation with $flaA_2$ and $flaA_3$. In transduction from the four *flaA* subgroups to 35 double mutants, the combination of $flaA_1 37$ and *motC fla-145*, of $flaA_2 23$ and 4 double mutants (*motC fla-111, -122, -127* and *-131*), and of $flaA_3 22$ and 5 double mutants (*motC fla-117, -118, -125, -140* and *-143*) showed no, or only a low grade of complementation. $flaA_2 34$ and $flaA_4 39$ did not complement or only partially complemented all 35 double mutants; however, these data are not reliable for assignment to complementation groups, because these *flaA* mutants for unknown reasons when used as donors evoke only few trails, even in crosses with *motC flaB* and *motC flaD*. Therefore, whether the remaining 25 mutants belong to the *flaA* subgroup or compose new cistron(s) is unknown.

In summary, most of the group I mutants (zero trail production) belong to *flaF*, the group II mutants (0.02–0.3% trail production) to *flaC* or *flaK*, and some of the group IV mutants (50–150% trail production) to *flaA, flaB* or *flaD*. *flaE* and *flaJ* are inferred to be perhaps subgroups of *flaK*.

Group I fla *(zero trail production) derived from* motA257 *and* motB275: Three double mutants were chosen from group I of *motA* and of *motB* (*motA fla-178, -198, -803, motB fla-155, -160* and *-165*). In transduction from these

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mutants to the six *fla* testers (*flaA41, B36, C38, D42, F25* and *K44*), they all complemented all the testers except *flaF25*. Probably the remaining group I mutants will also be found to belong to the *flaF* cistron.

Group II fla *(0.02–1% trail production) derived from* motA *and* motB: In transduction from the above six *fla* testers to 15 *motA fla*, 10 did not show complementation with *flaA41*, one (*motA fla-179*) with *flaB36* and the remaining four complemented all the testers. Thirteen of the 16 *motB fla* mutants did not show complementation with *flaA41*, two (*motB fla-169* and *-171*) with *flaB36*, and one (*motB fla-149*) with both *flaA41* and *flaB36*. To classify the *mot flaA* double mutants into subgroups of *flaA*, seven mutants were picked and transductions were carried out from them to the mutants of the *flaA* complementation subgroups and to *flaA41*; a deletion covering all the known *flaA* mutations. The results (Table 4) show that *motB flaA167* belongs to the same subgroup as *flaA$_1$37*, *motA flaA813* and *motB flaA157* to the subgroup of *flaA$_2$23* (or *flaA$_2$34*), and *motA flaA177* and *motB flaA156* to *flaA$_3$22*. *motA flaA804* and *motB flaA152* complemented all the *flaA* mutants except *flaA41*. These are thought to be new subgroup(s) of *flaA* and are tentatively termed as *flaA$_5$*.

Group III mutants (1–10% trail production) derived from motA *and* motB: Of seven double mutants of group III, two strains, SJ1837 and SJ1859, were excluded from the experiments because the former had been lysogenized with P22 and the latter had a leaky character on flagellation. The remaining five mutants (SJ1801, SJ1814, SJ1840, SJ1841 and SJ1848) complemented all the *fla* complementation testers. To test the linkage of the secondary mutation causing absence of flagella to *H1* (structural gene for phase 1 flagellin, determining also phase 1 antigen), mutant SJ1840, derived from *motA257* and with the (unexpressed) *H1* allele *H1-i*, was treated with phage grown on SJ697 (*mot$^+$ fla$^+$ H1-gp*). Swarms were streaked on NA medium and their H antigens were typed by slide agglutination tests with anti-gp, anti-i and anti-1.2 sera. Of 380 swarms tested, 357 (94%) expressed the *H1-gp* allele of the donor and 23 (6%) expressed the phase-2 antigen, 1.2. No swarm with antigen i was detected. As the frequency of cotransduction of *H1* with *motA* is less than 1% (ENOMOTO,

TABLE 4

Transduction from group III mutants of motA fla *and* motB fla *to the* flaA *subgroup mutants*

Recipient	Donor							
	motB fla-167	*motA fla-813*	*motB fla-157*	*motA fla-177*	*motB fla-156*	*motA fla-804*	*motB fla-152*	TM2
flaA$_1$37	1	3	3	3	3	3	3	3
flaA$_2$23	3	2	2	3	3	3	3	3
flaA$_2$34	3	1	1	3	3	3	3	3
flaA$_3$22	3	3	3	1	1	3	3	3
flaA$_4$39	3	1	1	2	2	3	3	3
flaA41	0	0	0	0	0	0	0	3

The grade of trail production is shown. Transduction procedures and abbreviations are the same as Table 2.

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unpublished data) this observation suggests the possibility that the secondary mutation in SJ1840 might be one preventing expression of *H1-i*, either by mutation within it or in its postulated operator, *ah1* (IINO 1969). If this were true SJ1840 though nonflagellate when in phase 1 would be flagellate when in phase 2. Strain SJ1840 and the four other double mutants were therefore streaked out on the NGA medium; in each case a few SD-type colonies, which are characteristic of nonmotile flagellate cells, were observed among the majority of colonies of LP morphology characteristic of nonflagellate cells (ENOMOTO and IINO 1963). The SD-type colonies expressed the phase 2 antigen, 1.2; overnight broth cultures of such colonies when streaked out yielded a minority of LP-type (nonflagellate) colonies. This phenomenon has been called O-H variation and is characteristic of *ah1* mutants of diphasic strains (IINO 1961). Further, transduction from each of the five double mutants to SW1061 (*ah1* mutant of TM2) on NGA medium containing anti-1.2 serum yielded no trails; i.e., there was no complementation of *ah1* and the secondary mutations of the five double mutants. These results indicate that all the double mutants of group III are *motA ah1* or *motB ah1*. The following mutant numbers were given to these strains; SJ1801 (*motB ah1–11*), SJ1814 (*motB ah1–12*), SJ1840 (*motA ah1-14*), SJ1841 (*motA ah1–15*) and SJ1848 (*motA ah1–16*).

Group IV fla *(50–150% trail production) derived from* motA *and* motB: Complementation tests with *fla* testers as recipients revealed that *motA fla-191*, *motB fla-148* and *-151* belonged to the *flaK* cistron and *motA fla-200*, *-807*, *motB fla-153*, *-159*, *-173* and *-175* to *flaC*.

In summary, the second mutation sites of group I mutants (zero trails) derived from *motA* and *motB* belonged to the *flaF* complementation group or undetermined, those of group II (0.02–1% trails) were classified into *flaA*, *flaB* and *flaAB*, group III (1–10% trails) to *ah1*, and group IV (50–150% trails) into *flaC* and *flaK*. Each group of double mutants was defined by the number of trails produced in transduction from the wild-type strain. This fact suggests that the number of trails might be dependent on the distance between the cistron of the secondary mutation site and *mot* site concerned. To test this inference, the order of the *mot* and *fla* cistrons had to be determined.

Mapping of mot *and* fla *genes:* The order *his—motC—H1—motB—motA* has been proposed by conjugation tests between Hfr and *mot* mutants (ENOMOTO 1966b), and the order *motC—H1—flaK—flaC* has been found by transduction tests using the double mutants, *motC flaK* and *motC flaC*, and by *H1*-cotransduction of *flaK* and *flaC* (ENOMOTO 1967). *flaF* is mapped between *gal* and *trp* by colicinoduction (SMITH and STOCKER 1962). It has been found that *motC* and *flaA* show cotransduction with *H1* at frequencies of 3–52% and 4–13% respectively (ENOMOTO 1966a; ENOMOTO and YAMAGUCHI 1969; IINO and ENOMOTO 1966), though the frequencies vary much with the donors used, and that *flaB* and *flaD* also show cotransduction with *H1* at low frequencies (PEARCE and STOCKER 1965; IINO and ENOMOTO 1966). *motA* and *motB* are probably adjacent because a deletion mutation extending through the whole of *motB* and a part of *motA* has been found (ENOMOTO 1966a). Recently the order *flaB*—

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flaD—flaA—H1 has been reported by JOYS and STOCKER (1969). *flaE* and *flaJ* probably form one complementation group together with *flaK* since they show a low grade of complementation with *flaK* (Table 3). Linkage relationships among *motA*, *motB*, *flaK* and *flaC*, and among *motC*, *flaA*, *flaB* and *flaD* are unknown.

Three-point reciprocal crosses among motC, flaA, flaB *and* flaD: It was found that a deletion mutant, *flaA41*, covering all *flaA* mutants so far detected, produces neither swarms nor trails in transduction with *motC244*, *272* and *279*, presumably because the deletion extends through *flaA* into *motC*. Accordingly *flaA* and *motC* are inferred to be contiguous. Assuming the order *flaB—flaD—flaA—H1* (JOYS and STOCKER 1969), this gives the order *flaB—flaD—(flaA, motC)—H1*. Experiments were carried out to test this order and to reveal the order of *flaA* and *motC*. If the order *flaB—flaD—flaA—motC* is correct, the number of wild-type recombinants (swarms) issuing from the reciprocal crosses between *flaA* and *motC flaD* will be very different because the cross with *flaA* as donor needs four crossovers to produce swarms whereas the cross with *motC flaD* as donor requires only two crossovers. Similarly, reciprocal crosses between *flaD* and *motC flaB* will show a difference. Transductions were therefore carried out between *flaA34* and *motC244 flaD50*, *flaD42* and *motC244 flaB114*. To test the transducing ability of each lysate, transduction to *motA257* was simultaneously carried out. As shown in Table 5 the number of swarms was about the same in reciprocal crosses of *flaA* with *motC flaD*. This makes the order *flaD—flaA—motC* unlikely; as *flaA* and *motC* are contiguous, the order must be *flaD—motC—flaA34*. In the cross of *flaD* with *motC flaB* the number of swarms differed by a factor of about 5 in the reciprocal crosses, as expected if the order is *flaB—flaD—motC*. Combining these data indicates the order *flaB—flaD—motC—flaA34*.

Three-point crosses involving ratio tests of H1: As described before the *flaA* complementation group can be divided into four subgroups by the grade of

TABLE 5

Three-point reciprocal crosses among flaA, B, D, *and* motC

Donor	Recipient	Number of swarms (percent)	Number corrected
flaA34	*motCflaD50*	375	1167
flaA34	*motA257*	120 (32.1)	
motCflaD50	*flaA34*	1600	1488
motCflaD50	*motA257*	402 (107.5)	
flaD42	*motCflaB114*	930	1016
flaD42	*motA257*	342 (91.5)	
motCflaB114	*flaD42*	3630	5386
motCflaB114	*motA257*	252 (67.4)	
TM2	*motA257*	374 (100.0)	

With the reciprocal crosses the number of swarms produced by 1 ml transduction mixture is shown. In the control crosses with *motA257* swarms per 0.1 ml mixture are scored. The correction was performed to make percentage of each donor 100.

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abortive transduction. To examine the order of *motC* and the *flaA* subgroups experiments were carried out with the following design: if the order *motC—flaA—H1* is correct, swarms produced by transduction from *flaA* to *motC* will express the *H1* allele of the donor at a lower frequency than that in the cross with the wild-type strain as donor; if the order *flaA—motC—H1* is correct the frequency will be approximately the same in transduction from *flaA* and from the wild type. Strain SJ1910 (which is a *motC244* mutant having *H1-a* introduced from *S. abortus-equi*, SJ241, by transduction) was used as a recipient and three *motB flaA* mutants chosen as representatives of $flaA_1$, $flaA_2$ and $flaA_3$ subgroups, as donors. *motB flaB*, parental *motB275* and TM2 were also used as donors for control experiments. Swarms on NGA medium were streaked on NA medium and their flagellar antigens were typed by slide agglutination tests with anti-i and anti-a sera. In this system the *motB275* locus of the donor is not relevant and the frequency was not significantly different when the donor was *motB275* or TM2 (Table 6). Donors *motB* $flaA_1167$ and *motB* $flaA_2157$ showed lower frequencies of cotransduction of donor *H1-i* by a factor of about 0.16, and *motB* $flaA_3156$ and *motB flaB169* showed approximately the same frequency as that of *motB275*. These data give the order (*flaB*, $flaA_3$)—*motC*—($flaA_2$, $flaA_1$)—*H1*. Considering the previously reported frequencies of cotransduction of *flaA* and *flaB* with *H1* ($flaA_137$, 13%, $flaA_223$, 10.8%, $flaA_322$, 4.1% and *flaB36*, 0.4%, IINO and ENOMOTO 1966), the order *flaB*—$flaA_3$—*motC*—$flaA_2$—$flaA_1$—*H1* is inferred.

Three-point reciprocal crosses among motA, motB, flaC *and* flaK: It has been known that *motA* and *motB* form contiguous cistrons since a deletion mutant, *motB292*, covers all of the *motB* mutations and some of *motA* (ENOMOTO 1966a). Using *motA246*, whose site is covered by the *motB292* deletion and is thus near the *motB* end of *motA*, and the two double mutants, *motB275 flaK148* and *motB275 flaC153*, reciprocal transduction tests were carried out (Table 7). In the cross with *motB flaK* the number of swarms (corrected for transduction ability of donor and of recipient) was less, by a factor of about 0.16, when the donor was *motA246*, than in the reverse cross. This indicates the order *flaK*—

TABLE 6

Two-point crosses of motC (H1-a) *with* flaA (H1-i) *and* flaB (H1-i), *involving ratio test of* H1

Donor	Recipient	Total swarms tested	Donor (i) type	Percent
motB275 $flaA_1167$	*motC244(H1-a)*	1105	18	1.6
motB275 $flaA_2157$	*motC244(H1-a)*	711	11	1.6
motB275 $flaA_3156$	*motC244(H1-a)*	692	59	8.5
motB275 flaB169	*motC244(H1-a)*	715	52	7.3
motB275(H1-i)	*motC244(H1-a)*	700	69	9.9
TM2*(H1-i)*	*motC244(H1-a)*	303	43	14.2

Swarms on NGA medium were streaked on NA and their H antigens were typed with anti-i and anti-a sera.

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TABLE 7

Three-point reciprocal crosses among motA, motB, flaC, *and* flaK

Donor	Recipient	Number of swarms	Number of swarms corrected	Ratio
motA246	*motBflaK148*	20	44.1	1
motBflaK148	*motA246*	41	260.0	5.9
motA246	*motBflaC153*	102	846.2	1
motBflaC153	*motA246*	254	1075.3	1.3

Swarms per 1.5 ml transduction mixture was scored. Correction was carried out to make the donor- and the recipient-transduction abilities described below 100. Transduction abilities of donors, which were calculated as percentages of the number of swarms produced in transduction from each to *flaD42* to that from TM2 to *flaD42*, are: *motA246* (83.1), *motBflaK* (33.9), and *motBflaC* (50.8). Transduction abilities of recipients, which are percentages of the number of swarms in transduction from TM2 to each recipient to that from TM2 to *flaD42*, are: *motA246* (46.5), *motBflaK* (54.7), and *motBflaC* (14.5).

motA—motB, since four crossovers are needed to produce swarms when *motA* is donor. In the similar cross between *motA246* and *motB flaC,* the corrected number of swarms was about the same in the reciprocal crosses; this argues against the order *motB—motA—flaC* and so, on the premise that *motA* and *motB* are adjacent, indicates the order *motA—motB—flaC*. Combining these two orders gives *flaK—motA—motB—flaC*. The order *motC—H1—flaK—flaC* has been inferred (ENOMOTO 1967). Combining it with the newly inferred order gives *motC—H1—flaK—motA—motB—flaC.* This order, however, is incompatible with *his—H1—motB—motA—leu* (ENOMOTO 1966b), inferred from linkage between *leu* and *motA* or *motB* in Hfr crosses; as *leu* is separated from the *motAB* region by about 40% of the Salmonella chromosome, this inference may well have been unreliable.

Cotransduction of motA, motB, flaC *and* flaK *with* H1: To test the order mentioned above, the frequency of cotransduction of *H1* with each cistron was examined using strain SJ697 (mot^+ fla^+ *H1-gp*) as donor. The transduction mixtures were streaked on NGA medium containing anti-i and anti-1.2 sera, corresponding to the flagellar antigens of phase 1 and phase 2 of the recipient; and on NGA medium containing only anti-1.2 serum. Swarms produced on the former medium must have the flagellar antigen gp of the donor and swarms on the latter medium may have either i or gp. The percent fraction of the number of swarms with antigen gp to that with i or gp is shown as cotransduction frequency in Table 8. Assuming all the *mot* and *fla* loci concerned to be on the same side of *H1* they indicate the order *H1—flaK—*(*motA, motB*)*—flaC.*

In conclusion, the gene order revealed by these experiments and prior work cited in the text is as follows: *his—*(*flaB, flaD*)—$flaA_3$—*motC*—$flaA_2$—$flaA_1$— (*H1, ah1*)—(*flaE, flaJ, flaK*)*—motA—motB—flaC—trp—flaF—gal.*

Dependence of trail production upon the distance between two cistrons: The foregoing experiments clarified the order of most of the *mot, fla* and *H1* genes. Based on this order, tests were made to see whether the number of trails produced by *mot fla, mot ah1* or *mot mot* double mutants in transduction from TM2

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TABLE 8

Cotranslation frequency of motA, motB, flaC, *and* flaK *with* H1

Donor	Recipient	Number of swarms with i or gp antigen	Number of swarms with gp antigen	Cotransduction frequency (Percent)
SJ697	*motA257*	275×10^2	61	0.22
SJ697	*motB275*	224×10^2	55	0.25
SJ697	*flaK48*	204×10	12	0.59
SJ697	*flaC38*	346×10^2	3	0.009

depends on the distance between the two mutated cistrons. Representative double mutants derived from each parental *mot* (*motA257*, *motB275* and *motC244*) were chosen and transductions were carried out with phage grown on TM2. Of the recipients used, *motA257 motC244* and *motB275 motC244* were obtained by transduction of the *motC244 ahl*$^+$ region into *motA257 ahl–14* and *motB275 ahl–11* (ENOMOTO 1969). Double mutants *motA flaD* and *motB flaD* were not obtained in this experiment. *flaD42* was used as a control throughout the transduction tests, because the trails it produces are distinct and easy to count and their number is, in fact, not different from that produced by *motA257*, *motB275* and *motC244*. The results are shown in Table 9; all the twenty-four double mutants except one can be divided into two groups by the number of trails they produce. The first group shows around 100% trail production in comparison with the control, the number of trails being 5 to 8 per 10^5 p.f.u. of the wild-type lysate. The second group shows less than 2% of the control value, the number of trails being less than 0.1 per 10^5 p.f.u. In the eight double mutants producing numerous trails, either both mutations are located in the *flaB*—(*H1 ahl*) segment (hereafter termed the left segment); or both mutations are located in the *flaK*—*flaC* segment (the right segment). By contrast in the fifteen double mutants producing only very few trails one mutation is located in the left segment and the other in the right. One mutant, *motC flaB114*, though both its mutations are situated in the left segment, showed a significantly low level of trail production, i.e., 22% of the control. This might be because the trails made by this mutant are too short to be reliably counted. Supposing that percentage of the trail production reflects the distance between the parental *mot* cistron and the secondary mutated one, the order of cistrons in Table 9 can be described as follows, where the figures in parentheses are the numbers of trails produced by double mutants as percent of the numbers produced by the control, single, mutant. For double mutants of *motA: flaB* (0.04); *motC* (0.20); *flaA$_3$* (0.32); *flaA$_2$* (0.53); *flaA$_5$* (0.63); *ahl* (1.38); *flaK* and *flaC* (*ca.* 100). For double mutants of *motB: flaB* (0.08); *flaA$_3$* (0.20); *motC* (0.24); *flaA$_2$* (0.44); *flaA$_5$* (0.54); *flaA$_1$* (1.06); *ahl* (1.86); *flaK, flaC* (*ca.* 100). These two orders agree except in respect of the relative position of *motC* and *flaA$_3$*. Similarly the order indicated for double mutants of *motC* is: *flaB* (22); *flaD, flaA$_3$, flaA$_2$* and *flaA$_1$* (all *ca.* 100); *flaK* (0.25); *flaC* (0.04). Combining the three orders indicated by frequency of trail production by double mutants gives: *flaB*—(*flaA$_3$, motC*)—

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TABLE 9

Dependence of trail production on the distance between two cistrons

Strain no.	Mutant no.	Trails/5 plates Expt. 1	Expt. 2	Total	%	Trails/10^5 pfu
SJ1864	$motAflaA_2813$	457	400	857	0.53	3.43×10^{-2}
SJ1828	$motAflaA_3177$	272	252	524	0.32	2.10×10^{-2}
SJ1855	$motAflaA_5804$	512	—	1028	0.63	4.11×10^{-2}
SJ1830	motAflaB179	48	20	68	0.04	2.72×10^{-3}
SJ1842	motAflaK191	945	909	1854×10^2	114.02	7.42
SJ1858	motAflaC807	906	1065	1971×10^2	121.22	7.88
SJ1909	motAmotC244	209	109	318	0.20	1.27×10^{-2}
SJ1840	motAahl-14	834	1415	2249	1.38	9.00×10^{-2}
SJ1818	$motBflaA_1167$	986	741	1727	1.06	6.91×10^{-2}
SJ1808	$motBflaA_2157$	387	329	716	0.44	2.86×10^{-2}
SJ1807	$motBflaA_3156$	188	134	322	0.20	1.29×10^{-2}
SJ1803	$motBflaA_5152$	438	—	876	0.54	3.50×10^{-2}
SJ1820	motBflaB169	93	42	135	0.08	5.40×10^{-3}
SJ1799	motBflaK148	1039	755	1794×10^2	110.33	7.18
SJ1804	motBflaC153	729	549	1278×10^2	78.60	5.11
SJ1907	motBmotC244	258	139	397	0.24	1.59×10^{-2}
SJ1801	motBahl-11	2066	1950	3016	1.86	1.21×10^{-1}
SJ374	flaD42	965	661	1626×10^2	100.00	6.50
SJ766	$motCflaA_1145$	452	878	1330×10^2	93.33	5.32
SJ728	$motCflaA_2111$	730	812	1542×10^2	108.21	6.17
SJ764	$motCflaA_3143$	578	848	1426×10^2	100.07	5.70
SJ732	motCflaB114	159	160	319×10^2	22.39	1.28
SJ741	motCflaD50	688	533	1221×10^2	85.68	4.88
SJ730	motCflaK48	188	167	355	0.25	1.42×10^{-2}
SJ716	motCflaC46	22	33	55	0.04	2.20×10^{-3}
SJ374	flaD42	680	745	1425×10^2	100.00	5.70

Transductions were carried out from TM2 to the double mutants under the conditions described in Table 2. Transductions with the double mutants of *motA* and *motB* were carried out simultaneously.

$flaA_2$—$flaA_5$—ah1—flaK—flaC, with *motA* and *motB* close to *flaK* and *flaC*. This agrees well with the order inferred from recombination frequencies mentioned in the foregoing paragraph, i.e., *flaB—flaD—$flaA_3$—motC—$flaA_2$—$flaA_1$—(H1, ah1)—flaK—motA—motB—flaC*. This agreement indicates that the number of abortive transductants produced by a double mutant is inversely related to the

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TABLE 10

Abortive transduction from the deletion mutant, flaA41, *to double mutants*

Donor:	TM2				*flaA41*			
	Trails/3 pl.				Trails/3 pl.			
Recipient	Expt. 1	Expt. 2	Total	Percent	Expt. 1	Expt. 2	Total	Percent
motAflaB179	19	22	41	0.02	2990	1371	4361	2.54
motAflaC807	1475	1514	2989×10^2	158.40	1118	561	1679×10^2	97.84
motAflaK191	1654	1486	4140×10^2	166.40	1088	495	1583×10^2	92.25
motBflaB169	98	46	144	0.08	2350	1509	3859	2.25
motBflaC153	966	738	1704×10^2	90.30	694	261	955×10^2	55.33
motBflaK148	1356	1014	2370×10^2	125.60	1078	417	1495×10^2	87.12
flaD42	999	888	1887×10^2	100.00	1092	624	1716×10^2	100.00

distance between its two mutated cistrons (provided this distance is short enough to permit some single transducing particles to enclose chromosome fragments carrying both loci). Thus, the frequency of abortive transductants to motility, i.e., of trails whose production does not require recombination, can be used for mapping.

Tables 10 and 11 show the results of abortive transduction using two deletion mutants, *flaA41* and *flaB292,* as donors. The former covers all the *flaA* subgroups and *motC,* and the latter covers all of *motB* and a part of *motA.* The frequency of trail production by *motA257 flaB179* increases 127-fold (from 0.02% to 2.54% of the control) when the donor is *fla41* instead of the wild type, and the frequency for *motB275 flaB169* is increased 28-fold (from 0.08% to 2.25% of the control) (Table 10). In these two instances the deleted region of the donor lies between the mutated loci of the recipient and the shortening of the distance between the two wild-type alleles in the donor will therefore be expected to increase the frequencies of the rare class of transducting particles carrying both loci. As shown in Table 11 the frequency of trails by *motC244 flaC46* was similarly increased three-fold (from 0.03% to 0.09% of the control) when the donor was the deletion mutant *motB292:* this result also is to be expected, since the region deleted in *motB292* lies between *motC* and *flaC.* Furthermore when the recipient was *motC244 flaK48* the number of trails was about 4.5-fold greater (0.15% to 0.68% of the control) when the donor was *motB292* instead of the wild type, even

TABLE 11

Abortive transduction from the deletion mutant, motB292, *to the* motCfla *double mutants*

Donor:	TM2				*motB292*			
	Trails/3 pl.				Trails/3 pl.			
Recipient	Expt. 1	Expt. 2	Total	Percent	Expt. 1	Expt. 2	Total	Percent
motCflaA111	....	1097	2194×10^2	86.96	1197	553	1750×10^2	77.64
motCflaC46	49	33	82	0.03	129	64	193	0.09
motCflaK48	200	188	388	0.15	840	699	1539	0.68
flaD42	1365	1158	2523×10^2	100.00	1524	729	2253×10^2	100.00

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though the *motB292* deletion is not between *motC* and *flaK*. This phenomenon will be referred to in the DISCUSSION.

DISCUSSION

The gene order *his—flaB—flaD—*$flaA_3$*—motC—*$flaA_2$*—*$flaA_1$*—(H1, ah1)—flaK—motA—motB—flaC—trp* has been inferred by complete transduction tests. In the reciprocal three-point crosses carried out to check the order *flaB—flaD—motC—*$flaA_2$ (Table 5), the cross between *motC flaD* and *flaB* has not been performed, because of the high reversion rate of *flaB36*. One *fla* mutant, *fla-149*, isolated from *motB275*, complemented neither *flaA41* nor *flaB36* though it did complement *flaD*. If *fla-149* has a multisite mutation reaching from *flaA* into *flaB* the order *flaB—flaD—(motC, flaA)* cannot be correct. Deletion mapping has revealed that in *Salmonella abortus-equi* the *flaB* cistron is located between *flaD* and *flaA* (YAMAGUCHI, personal communication). The *flaB* mutants used in this work should be re-examined with the deletions of *S. abortus-equi*. It is perhaps relevant that *flaB36* (used in this experiment as a representative of *flaB*) complements *fla-50* and *fla-59* (VARY and STOCKER, personal communication), the mutants which defined complementation group B of JOYS and STOCKER (1965); one of these mutants, *fla-50*, was used in the crosses which indicated the order *flaB—flaD—flaA—H1* (JOYS and STOCKER 1969). The order *H1—motA—motB* (Table 7) now inferred is the reverse of the order *H1—motB—motA* previously inferred from an Hfr cross (ENOMOTO 1966b). The cotransduction frequency with *H1* does not significantly differ between *motA* and *motB* (Table 8) and the number of abortive transductants observed from *motA motC* and *motB motC* double mutants suggests the order *(motC, H1)—motB—motA*. For these reasons the order of *motA* and *motB* remains uncertain. In the three-point crosses shown in Table 7 the number of transductants produced by some of the double mutants in transduction from TM2 is less than 15% of the control and the data are corrected to make these percentages as well as the transducing ability of lysates 100. Such experiments are usually carried out with recipients which produce nearly the same number of transductants in crosses with the wild-type strain. The correction for recombination ability of the recipients might make the data inaccurate. Taking these uncertainties into account the order indicated by the present and previous results is *his—(flaB, flaD)—*$flaA_3$*—motC—*$flaA_2$*—*$flaA_1$*—(H1, ah1)—flaK—(motA, motB)—flaC—trp*.

Based on the above order a new fact has been revealed: the number of trails which are produced by the double mutants, *mot fla, mot mot* and *mot ah1*, in abortive transduction from TM2 is in inverse proportion to the distance between the two cistrons of the double mutants. This is useful for genetic mapping as a new tool. However this effect is only observed when the distance between the two mutated loci of the double mutant is greater than some critical length. The number of trails produced by a double mutant may then be less than 2% (*motB ah1*, Table 9), so far as tested with double mutants isolated in this experiment, of the yield from a single mutant or closely linked double mutants. OZEKI (1959)

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reported that the number of abortive transductants produced by a double mutant (*trp cysB*) in transduction with a wild-type donor was approximately the same on minimal medium as on medium containing either cysteine or tryptophan. It may be that when two loci are closely linked and all the transducing fragments carrying the one locus also can carry the other. A low frequency of trail production by the double mutants in this experiment indicates that only a small fraction of the transducing particles which carry one of the two loci carry also the other. The following discussion is concerned with the production of such a minority of transducing particles.

The genetic constitution of the transducing fragment must be determined by the length of the fragment packed in a particle and the manner of cutting of the host chromosome to produce the fragments. It has been found in a P1-*Escherichia coli* system that most or all of the transducing particles contain only fragments of the bacterial chromosome and that the fragments have the same molecular weight as a phage genome (IKEDA and TOMIZAWA 1965a); and also that the length of the transducing fragment as well as phage genome is determined by the size of the phage particle, that is, capacity of the phage head (IKEDA and TOMIZAWA 1965b). The DNA of infective particles of several phages shows a unimodal size distribution in sucrose gradient centrifugation and a definite contour length with only a small standard deviation of 2–6% on electron micrographs (THOMAS 1966; LANG *et al.* 1967; RHOADES, MACHATTIE and THOMAS 1968). From these data the length of the transducing fragments is inferred to be determinate. As to the manner of cutting, whether it is random or not, several data indicate that some degree of heterogeneity exists as to the termini of the fragments carrying a given genetic marker (ROTH and HARTMAN 1965; PEARCE and STOCKER 1965; ENOMOTO 1967; EISENSTARK, EISENSTARK and CUNNINGHAM 1968), though these data do not deny the termination of most of the fragments at a certain preferential point (OZEKI 1959; PATTEE *et al.* 1968). Accordingly, at present the following two possibilities should be considered as to the formation of the transducing fragments: fragments with a standard length produced by cutting either at preferred positions or at random position(s). It is unknown in both models whether both ends of the fragment are cut before its incorporation into a phage particle. The production of a minority of transducing particles containing a locus absent from the majority class, mentioned above, can be explained by either model of fragmentation if the length of the fragments is slightly variable within the permissible range, for instance, normally distributed with a small standard deviation. In this case the lengths of the fragments presumably describe a normal distribution curve. When the two genes concerned with abortive tranduction are situated with respect to each other at a distance slightly greater than the farthest possible distance for the fragment with mean size to carry, most of the transducing particles cannot carry both genes simultaneously, whereas a few particles having a slightly larger capacity for genetic material can do it, constituting a minority of the transducing particles. In the case of the fragment formation by regular cut at preferred points, if one of the two genes is located just outside the fragment that the particle of mean size can carry, the above phenomenon is explainable

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because slightly larger particles will carry both genes. In transduction from the deletion mutants some double mutants showed an increased frequency of trail production when the deleted region of the donor lies between the two loci of the double mutant (Table 10 and 11), as would be expected on either model of production of transducing fragments. However in transduction from the deletion, *motB292*, to the *motC flaK48* double mutant, the trail production also increased to 4.5 times of the control (Table 11), though the deletion in *motB292* does not extend into the region between *motC* and *flaK*. What explanation can be given for this phenomenon? If the transducing fragments are formed by random cuts or if both ends of the fragment are cut at preferred points, such increment would not be expected. The most plausible explanation is that the host chromosome is cut at certain preferred points, to produce the fragments larger than the transducing fragment itself and that the transducing fragments are then measured off from one (or both) end(s) of this large fragment in a definite or in a random direction. In this case the large fragment carries at least the region *motC*—*flaK*—(*motA*, *B*)—*flaC* and the direction in measuring off is probably definite, from right to left in the sequence ← *motC*—*flaK*—(*motA*, *B*)—prefer site of cut, so that the deletion in *motB292* will make the fragment carrying *motC* and *flaK* increase. If *motC* lies just to the left of the left-hand end of the *flaK*-carrying fragments of standard length cut from the wild-type chromosome because of the preferred site of cutting to the right of *motB*, then only the small number of fragments of greater than standard length will carry both *flaK* and *motC* in the case of a lysate of the wild-type strain. The effect of the deletion in *motB292* will be to move the position of the left-hand terminus of fragments of standard length to the left by a distance equal to the length of the deletion, and therefore closer to *motC*. In consequence, if fragment length is normally distributed, the fraction of fragments carrying *flaK* as well as *motC* will be increased in phage grown on the *motB292* deletion mutant, as observed.

SUMMARY

A number of nonmotile double mutants, mot^- fla^-, mot^- mot^- and mot^- $ah1^-$, have been isolated from three parental *mot* mutants, $motA^-$, $motB^-$, and $motC^-$, of *Salmonella typhimurium* and used for mapping relative positions of *mot*, *fla* and *H1* genes. The inferred order is as follows: *his*—(*flaB*, *flaD*)—$flaA_3$—*motC*—$flaA_2$—($flaA_5$, $flaA_1$)—(*H1*, *ah1*)—(*flaK*, *flaE*, *flaJ*)—(*motA*, *motB*)—*flaC*—*trp*. The number of abortive transductants issuing from the double mutants in transduction with the wild-type strain is in inverse proportion to the distance between two mutations. In this case one mutation should be located between *flaB* and *ah1* (left side) and the other between *flaK* and *flaC* (right side), and the number of abortive transductants is very few as compared with that produced by the double mutants whose two mutations are together located in either the left side or the right side. This phenomenon is useful for genetic mapping as a new tool. How the transducing fragments arise from the bacterial chromosome is discussed.

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